A NOTE ON THE SIMULTANEOUS ESTIMATION OF MINUTE QUANTITIES OF SULPHUR AND PHOSPHORUS.*

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In 1910, Wolf and Österberg[1] described a method in which the substance is oxidized with a large excess of nitric acid in a Kjeldahl flask, and then evaporated to dryness in the usual manner with Benedict's reagent. After ignition over a flame, the residue is taken up in dilute hydrochloric acid and barium sulphate is precipitated and weighed. The excess of barium is removed from the filtrate and phosphorus determined by Neumann's method.

We carried out a few experiments, modifying this method in order to observe if it would be possible to utilize benzidine as the precipitant. In analyzing an organic substance containing 1% of sulphur, the use of a 1.0 gram sample will yield 73 mg. of barium sulphate. If the quantity taken is 0.20 Gm. (= 2 mg. sulphur) the barium sulphate weighed would amount to 0.0146 Gm. If benzidine could be used, one would require for 2 mg. sulphur 6.25 cc $N/50$ alkali to neutralize the benzidine sulphate (1 cc $N/50$ = 0.00032 Gm. sulphur) Raiziss and Dubin[2] have described a method which involves titrating the benzidine sulphate with $N/10$ $KMnO_4$ solution. Here 1 cc $N/10$ $KMnO_4$ is equivalent to 0.001 g. sulphur, making it about three times as delicate as the regular titration with alkali.

Since the methods for the estimation of phosphorus in quantities from 0.1 to 1.0 mg. are well known, it appeared that we might be successful in the determination of both sulphur and phosphorus even if very small samples were employed. MacArthur[3] describes a method for the analysis of the lipin extract of tissues, in which the material approximately (0.08 Gm.) is fused with 5 cc of a 20% solution of potassium hydroxide and sodium nitrate (4–1). Of the final solution 1/5 (0.016 Gm.) served to estimate sulphur by the benzidine method. This method is further discussed but no experimental work is given to show what accuracy might be expected with it.

It seemed needless in our work to take such small quantities as those mentioned by MacArthur. Furthermore the known proved methods do not appear to justify them.

The work described in this paper was carried out for the purpose of determining (1) the accuracy with which 1 mg. of organic sulphur might be determined; (2) the effect of the method employed on the subsequent application of Raper's method on the filtrate.

It was found that, when the alkaline fusion method (Na_2O_2) is employed, that the use of the regularly required amount of fusion mixture prevents the quantitative precipitation of benzidine sulphate. The use of Benedict's solution in place of the fusion mixture also fails to permit the use of benzidine, although the error is much smaller. One must, therefore, rely on the barium sulphate precipitation, as first suggested by Wolf and Österberg. We prefer the fusion method for the analysis of lipins.

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[1] *Biochem. Zeitschrift*, 29, 429, 1910.

[2] *J. Biol. Chem.*, 18, 300, 1914.

[3] *Jour. Am. Chem. Soc.*, 41, 1232, 1919.

Raper's method was not unfavorably influenced by this preliminary determination of sulphur.

EXPERIMENTAL.

The material analyzed was casein. It was first analyzed for phosphorous by the regular fusion method, *i. e.*, 1 Gm. casein + 5 g. Na_2CO_3 + 5 Gm. Na_2O_2 with subsequent addition of 5 Gm. Na_2O_2. The solution was acidified with nitric acid, concentrated to a volume of 200 cc. The molybdate was precipitated as usual and the phosphorus weighed as $Mg_2P_2O_7$. Found, P = 0.80, 0.80%. The above analysis was repeated by Neumann's method.[1] Found, 0.76, 0.75%.

A quantity 0.2 Gm. (= 1.6 mg. P) was ashed by the regular procedure, using 10 cc of nitric + sulphuric acids (1–1). Five cubic centimeters was first added, and the mixture boiled until free from the oxides of nitrogen. Then 3 cc was added, and the mixture boiled further. Then 2 cc further was added and the mixture boiled to a volume of 4 cc. Water (20 cc) was added and the mixture boiled for ten minutes. The solution is made up to 100 cc using 24 cc of ammonium nitrate solution (190 Gm. in 300 cc). The solution is heated to 80° and precipitated with 8 cc of 10% ammonium molybdate solution. The solution is shaken and held in the bath for 20 minutes, with occasional shaking. The mixture is cooled in the ice-bath, 8 cc of 50% alcohol is added, and then filtered through a 7 cm. filter. The precipitate remaining in the flask is washed out by means of ice cold 50% alcohol and the precipitate on the filter paper washed with dilute alcohol until the washings are free from acid, 7 to 8 washings usually sufficing.

The precipitate is then dissolved on the filter paper, by dropping N/20 sodium hydroxide solution on it, from a burette, care being taken to have an excess not exceeding 2 or 3 cc. The filter paper is thoroughly washed with water until free from alkali. The volume should be about 175 cc. The solution is boiled down to 100 cc to remove ammonia. Phenolphthalein test solution (0.5 cc) is added and the solution titrated with *N*/20 sulphuric acid solution, 2 cc excess being added. The solution is then boiled to remove carbon dioxide. After removal of CO_2, the solution is titrated to a light pink color with *N*/20 alkali. (1 cc *N*/20 sol. = 0.0553 Mg. phosphorus.)

When 0.10 Gm. casein was taken, and the entire operation conducted proportionately the same result was obtained.

By Raper's[2] method, using 0.2 Gm. samples, we obtained 0.81% P.

Sulphur Determinations.—Several estimations were made by the regular fusion method using 1.0 Gm. samples. Found: 0.69, 0.71, 0.68%.

Effect of Benedict's Reagent on Precipitation of Benzidine Sulphate.—A standard solution of K_2SO_4 was prepared by dissolving 5.4438 Gm. in 1 liter. (1 cc = 1 mg. S.) This solution was diluted 1:10 for the following work, so that 10 cc = 1 mg. S.

Fifteen cc (1.5 mg. S) was transferred to a wide mouth flask. The solution was acidified with normal hydrochloric acid. Benzidine hydrochloride solution[3] (25 cc) is added and a few cubic centimeters of acetone. After standing, the precipitate was filtered on a Gooch, washed with ice cold 50% acetone, and then titrated (*a*) with *N*/50 sodium hydroxide[4] and (*b*) with an approximately *N*/10 potassium permanganate solution.

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[1] Kleinmann, *Biochem. Zeits.*, 99, 95, 1919.

[2] *Biochemical Journal*, 8, 649, 1915.

[3] Made by dissolving 6.7 Gm. benzidine in 29 cc hydrochloric acid (1.12) and diluting to 1000 cc.

[4] *J. Biol. Chem.*, 47, 63, 1921.

Taken.	Cc N/50 NaOH.	Cc $KMnO_4$ used.	1 cc $KMnO_4$.
1.5 mg. S	4.5	13.85	0.0001083 Gm. S
1.5 mg. S	4.5		
1.0 mg. S		9.0	0.000111 Gm. S
		8.9, 9.1	

The above work was repeated, first evaporating the solution to dryness, and oxidizing with 5 cc Benedict's reagent. After igniting and taking up in hydrochloric acid (1:4) the solution was almost neutralized and the benzidine precipitation carried out as before. The following results are typical.

Sulphur taken.	Cc N/50 alkali.	Cc $KMnO_4$ used.	Error.
1.0 mg.	2.8	8.3	−8%
1.5 mg.	4.5	13.65	
	4.45	13.8	
	4.4	13.9	
	4.35	12.9	−7%

The use of Benedict's reagent in amounts of 5 cc per 1 mg. of sulphur gave results which while not sharp were tried on Casein. This substance was boiled with 5 cc HNO_3, then 3 cc fuming acid was added and finally 2 cc. After boiling, the solution was transferred to a small casserole and evaporated to dryness with 5 cc Benedict's reagent. (Less cannot well be used.) The analysis was completed as above described.

Casein taken.	Cc $KNnO_4$ required.	S found mg.	% Sulphur.
0.2000	12.5	1.35	0.68
	12.9	1.40	0.70
	12.45	1.35	0.675
	12.1	1.31	0.66
	12.15	1.32	0.66
	12.3	1.35	0.665
	13.3	1.44	0.72

These results[1] indicate the accuracy which may be expected under these conditions, but they are less accurate when more Benedict's solution is required, or when less sulphur is present. Furthermore in the analysis of lipins there is a greater tendency to loss by puffing than with casein.

When the analysis is carried out exactly as before except that $BaSO_4$ is precipitated and weighed, 1.29 to 1.32 mg. sulphur was found, *i. e.*, 0.65–0.66%.

As a result of our experience we prefer the fusion method. 0.2 Gm. was fused with 1.0 Gm. Na_2CO_3 + 1. Gm. Na_2O_2 and then a second 1. Gm. Na_2O_2 was added. By this method 1.37 mg. sulphur (0.685%) was found.

Using the regular fusion method, we found that benzidine cannot be employed as the precipitant when a requisite amount of alkali is used.

Determination of Phosphorus in Filtrates.—The influence of copper or the sodium salts is negligible. The excess of benzidine or barium was removed with sulphuric acid and 5 cc conc. H_2SO_4 added to filtrate. The analysis when com-

[1] Occasionally the variation exceeds that shown in this table owing to the failure of the benzidine sulphate to crystallize. In these analyses the precipitation mixture stood in the ice-chest over night. For example the duplicate with analysis 5 required only 11.05 cc $KMnO_4$ (0.60%). A number of attempts were made to secure more perfect precipitation through the addition of 10 cc standard K_2SO_4 solution, just before adding the benzidine. Results as low as 0.61% have been recorded with this modification.

pleted by Raper's method gave 0.80, 0.82, 0.775, 0.78, 0.74, 0.81,[1] 0.80,[1] 0.78, 0.81%.

SUMMARY.

Small quantities of sulphur (1 mg.) in organic substances can be determined by the fusion method, by weighing barium sulphate as accurately as with the benzidine method, using Benedict's reagent. For lipins the fusion method is preferable because the chance of loss by puffing is eliminated.

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THE BIOLOGICAL TESTING OF SALVARSAN AND ITS DERIVATIVES.*

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In the armamentarium of the modern physician certain compounds of arsenic have a very important position. This position has been gained by the clinical and experimental evidence which has accumulated during the past eighteen years since the announcement of the discovery of Salvarsan by Ehrlich. While compounds of arsenic were used prior to this and hundreds have been synthesized and tried in the treatment of disease since then, none have equalled Salvarsan and Neosalvarsan for use in the treatment of syphilis.

The properties which the physician seeks are purity, complete solubility, relative non-toxicity and therapeutic efficiency. These properties are controlled by the manufacturer in his laboratories before the product is offered to the physician. For this purpose he maintains a staff of chemists and biologists, the first to conduct the chemical and physical tests, the second to test the product for its toxicological and therapeutic properties by the modern scientific methods which have been developed for these purposes. These methods are the result of the investigations of workers in Government and private laboratories both in this country and abroad. The early work of Ehrlich and Hata in Germany has been followed by that of Dale in England, Danysz in France and Voegtlin and others in the United States. This laboratory has carried on such biological tests since the advent of Salvarsan as an American made product. It is this type of studies that has given Salvarsan and Neosalvarsan uniform reliability without sacrificing therapeutic activity. Another factor contributing to the uniformity of these products is their manufacture on a large factory scale rather than a number of small lots such as are made in smaller laboratories.

In order that a compound shall have value in the treatment of a disease it is necessary that it be tolerated by the animal body in a much larger amount than is required to kill the micro-organism which is the causative factor in this disease. It is obvious that if quinine were tolerated by the plasmodium of malaria in larger amounts than can be borne by man, quinine would never have been used in the treatment of this infection. So, in the field of organic arsenicals, those compounds are discarded which have a curative dose approaching the tolerated

[1] 0.1 Gm. samples.

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